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Total Synthesis of Cytotoxic Macrolide Amphidinolide B₁ and the Proposed Structure of Amphidinolide B₂

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Amphidinolide B₁ (**1**) and its C₁₈ epimer amphidinolide B₂ (**2**) have generated significant attention from the synthetic community^{1,2} because of their potent cytotoxic activity against several cancer cell lines. In fact, macrolide **1** is the most potent member of this family with a reported IC₅₀ value of 0.14 ng/mL against L1210 murine leukemia cell line.³ Despite these efforts, their total syntheses remain elusive targets. Macrolides **1** and **2** represent considerable synthetic challenges because of their highly substituted C₁₃–C₁₅ diene functionality,^{1c,4} the densely functionalized C₂₁–C₂₆ right-hand portion, the labile C₆–C₉ epoxy alkene, and a 26-membered lactone. Our retrosynthetic strategy starts by disconnection at C_{8,9} to reveal the aldehydes **3** and **4** (Scheme 1). Further cleavage of the ester linkage at C₂₅ would lead to the alcohols **5** and **6** and the keto phosphonate **7**. The C₁₈–C₁₉ bond should be available via a diastereoselective aldol reaction with the aldehyde **8** and α -oxy ketone **9**. We have previously reported a chelation strategy for the synthesis of the 18*S* stereochemistry present in amphidinolide B₁^{1b,c,5} and this approach has been used by others in the field,⁶ including Fürstner's recent total syntheses of amphidinolide G and H.^{6a} Herein, we report a nonchelation strategy for construction of the C₁₈ stereochemistry and its application to the total syntheses of cytotoxic macrolide amphidinolide B₁ (**1**) and the proposed structure of amphidinolide B₂ (**2**).

Our syntheses of the aldehyde and methyl ketone subunits commenced with the previously reported intermediates **11** and **14** (Scheme 2).^{1a,c} Conversion of the C₉ nitrile **11** into the corresponding acetate **12** followed by selective functionalization of the C_{18,19} alkene using AD mix β^* provided the C_{18,19} diol as an inconsequential 6:1 mixture of diastereomers.^{1c} Cleavage of the resultant diol with sodium periodate provided the desired aldehyde **8**. For the synthesis of the Eastern subunit **9**, Horner–Wadsworth–Emmons olefination of aldehyde **14** followed by dihydroxylation yielded the diol **16**. Next, bis-silylation followed by selective C₂₅ TES deprotection yielded the free alcohol at C₂₅. Finally, Mitsunobu inversion of the alcohol followed by saponification and TMS protection revealed the ketone **9**.

The key diastereoselective aldol coupling is shown in Scheme 3. Treatment of ketone **9** under our standard LDA, Et₂O/THF conditions that proved effective with the C₂₁ OPMB series^{1c} resulted in low conversion and poor diastereoselectivity favoring the 18*S* stereochemistry [approximately 1.5:1 dr (**5**:**6**)]. Interestingly, addition of TMEDA led to a dramatic rate acceleration and a reversal of the selectivity [2:1 dr (**6**:**5**)]. Additional cooling of the reaction to –100 °C led to improved diastereoselectivity [8:1 dr (**6**:**5**)] in reasonable yield (65% overall). While we are still exploring the nature of the diastereoselectivity, one possible explanation could be a transition state which minimizes the dipoles of the C₂₁ C–O σ bond and the enolate.⁷ The alternate 18*S* diastereomer can be obtained by performing the reaction at higher

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Supporting Information Available: Complete experimental procedures are provided, including ¹H and ¹³C spectra, of all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

temperatures (-40°C , 1.2:1 dr **5:6**). Silyl protection of alcohols **5** and **6** separately using TESCl/DMAP yielded the silyl ether compounds **17** and **18**, respectively.

With an effective strategy for the coupling of the Eastern and Western subunits in hand, we turned our attention to the key macrocyclization event (Scheme 4). We chose to initially explore this sequence on the 18R compound **18**. TMS deprotection of silyl ether **18** at C₂₅ and incorporation of the C₁–C₈ subunit provided the keto phosphonate **20**.⁸ Removal of the C₉ acetate followed by Ley's TPAP oxidation revealed the target aldehyde **4**. The corresponding aldehyde **4** proved to be highly reactive as attempted isolation led to considerable loss of material. Interestingly, significant amounts of the aldehyde **4**, formed during the TPAP oxidation, appeared to undergo *spontaneous intramolecular Wadsworth–Emmons olefination* to provide the desired macrocycle **22**. The conversion could be driven to completion by the addition of LiCl and Hunig's base⁹ in 51% yield. A similar sequence was followed for construction of the 18S macrocycle **21**. In this case, Ba(OH)₂ proved more effective for driving the macrocyclization to completion. Additionally, we were pleased to observe that macrocycle **21** crystallized upon standing—allowing us to confirm the stereochemistry in **21**.¹⁰

With an efficient route into the macrocycles **21** and **22**, the final challenges were the incorporation of the C₆–C₉ allylic epoxide moiety and deprotection. As before, we explored the initial chemistry on the 18R macrolactone **22** (Scheme 5). Our previous experiences with deprotection of late stage amphidinolide B intermediates made us mindful of the difficulty of the final deprotection sequence.^{1d} Regioselective and stereoselective reduction of the C₇ carbonyl functionality could be accomplished with the (*S*)-CBS reagent.¹¹ We next had intended to epoxidize the alkene under Sharpless conditions;¹² however, the presumed steric congestion of the C₇ alcohol thwarted this approach. Fortunately, use of Ti(Oi-Pr)₄ and TBHP in the *absence* of DIPT led to formation of both the *syn* and *anti* diastereomer—favoring the *syn* stereochemistry (2:1 dr).¹³ As the *syn* diastereomer **24** contained the proposed stereochemistry in amphidinolide B₂, we initially proceeded forward with that diastereomer. Selenide incorporation¹⁴ and elimination under our recently developed TPAP/NMO conditions^{1d,15} yielded the fully functionalized macrocycle. Unfortunately, all attempts to remove the silyl protecting groups under fluoride or acidic conditions led to decomposition. Suspecting that the allylic epoxide might be the culpable functionality, we next explored global deprotection on the epoxy selenide **26**. We were quite pleased to find that treatment with TAS-F^{6a} cleanly removed all silyl protecting groups to provide the polyol **28**. The final selenide oxidation/*syn* elimination proved problematic under standard (H₂O₂) conditions. This issue was not completely surprising as we have previously encountered this problem in our azaspiracid work¹⁵ as well as a previous generation amphidinolide approach.^{1d} We have attributed this deleterious reactivity to the α -hydroxy ketone moiety. Although our TPAP/NMO conditions¹⁵ are not compatible with the polyol functionality of **28**, we were gratified to find that bistrimethylsilylperoxide (TMSOOTMS) cleanly facilitated selenide oxidation with in situ *syn* elimination to reveal product **2**. Surprisingly, this compound **2** *did not match* with the spectra data provided for the natural product amphidinolide B₂.³ We followed a similar sequence to the C_{8,9} epoxide diastereomer **30** which too did not correspond with the reported data for amphidinolide B₂. In both cases, the ¹H NMR shift for the H₁₄ alkene was shifted significantly downfield as compared to the natural product data. Careful inspection of the isolation paper revealed that the stereochemical analysis of amphidinolide B₂ was based primarily on the differences in the ¹H NMR in the C₁₇–C₁₉ region of the natural product as compared to amphidinolide B₁ (**1**).^{3b} It is important to note that Shimizu and Clardy obtained X-ray crystallographic structure of natural product **1**.^{3b} It is clear from our work that the structural differences between amphidinolide B₁ and B₂ are more complicated than initially expected. *On the basis of this information, we have concluded that the proposed structure of amphidinolide B₂ is incorrect.*

Next, we shifted our focus to the total synthesis of amphidinolide B₁ (**1**) (Scheme 6). We applied an analogous strategy for the synthesis of **1** as was described for the 18*R* series. It appears that a slight reversal in selectivity in the epoxidation occurs with the 18*S* stereochemistry—now with a modest preference for the undesired C_{8,9} epoxide. Fortunately, these diastereomers are chromatographically separable. Conversion of syn epoxide **32** to the selenide followed by TASF deprotection yielded the penultimate intermediate. Finally, we were grateful to find that tandem selenide oxidation/elimination using our bis-TMS peroxide conditions yielded the natural product amphidinolide B₁ (**1**). The synthesized material **1** matched with the spectra data reported by Kobayashi and co-workers for amphidinolide B₁.^{3c}

In summary, the first total syntheses of amphidinolide B₁ and the proposed structure of amphidinolide B₂ have been achieved. The longest linear sequence is 25 steps from commercially available lactic acid. Determination of the actual structure of amphidinolide B₂ will be reported in due course.

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Scheme 1.
Retrosynthetic Analysis of Amphidinolide B₂

Scheme 2. Synthesis of Western and Eastern Subunits^a

^a (i) DIBAL-H, PhMe; (ii) NaBH₄, EtOH/CH₂Cl₂ (1:1), 85% (two steps); (iii) Ac₂O, pyr., CH₂Cl₂, 94%; (iv) AD Mix β*, *t*-BuOH, H₂O, 88%, 6:1 dr; (v) NaIO₄, THF, H₂O, 99%; (vi) **15**, NaH, DME, 76%; (vii) K₂OsO₂·4H₂O, (DHQ)₂PHAL, K₆Fe(CN)₃, MeSO₂NH₂, K₂CO₃, *t*-BuOH, H₂O, 77%, 10:1 dr; (viii) TESOTf, 2,6-lut., CH₂Cl₂, -78 °C, 92%; (ix) HOAc: THF: H₂O (8:8:1), 0 °C, 89%; (x) DEAD, Ph₃P, 4-NO₂-C₆H₄CO₂H, THF, 82%; (xi) then Ba (OH)₂·8H₂O, MeOH, 0 °C, 72%; (xii) TMSOTf, 2,6-lutidine, CH₂Cl₂, -78 °C, 95%.

Scheme 3. Key Diastereoselective Aldol Coupling^a

^a (i) LDA, TMEDA, Et₂O, THF, -100 °C then add **8**, 65% (8:1 dr, **6:5**); (ii) LDA, TMEDA, Et₂O, THF, -40 °C then add **8**, 66% (1.2:1 dr, **5:6**); (iii) TESCl, DMAP, CH₂Cl₂, room temp.

Scheme 4. Closure of the Macrocycle^a

^a (i) HOAc/THF/H₂O (8:8:1), -10°C; (ii) **5**, Cl₃C₆H₂COCl, Et₃N then DMAP, PhMe; (iii) Ba(OH)₂·8H₂O, MeOH, H₂O, 89% for **19**, 91% for **20**; (iv) TPAP, CH₂Cl₂ then Ba(OH)₂·H₂O, THF, H₂O, 0.01 M final concentration, 51% of **21**; (v) TPAP, CH₂Cl₂ then LiCl, *i*-Pr₂NEt, MeCN, 0.01 M final concentration, 50% of **22**.

Scheme 5.

Synthesis of Proposed Structure of Amphidinolide B₂ and its C_{8,9} Diastereomer^a

^a (i) (*S*)-CBS, BH₃·DMS, THF, −20 °C, 3 h, >10:1 dr, 67%; (ii) Ti(*Oi*-Pr)₄, TBHP, CH₂Cl₂, −20 °C, 5 h, mol. sieves, 74%, 2:1 dr (**24**:**25**); (iii) Bu₃P, *o*-NO₂C₆H₄SeCN, THF; (iv) TAS-F, DMF/THF/H₂O (10/1/0.02), 0 °C; (v) TMSOOTMS, NaHCO₃, CH₂Cl₂.

Scheme 6. Total Synthesis of Amphidinolide B₁^a

^a (i) (*S*)-CBS, BH₃·DMS, THF, −30 °C, 3 h, 3.6:1 dr, 74%; (ii) Ti(O*i*-Pr)₄, TBHP, CH₂Cl₂, −20 °C, 5 h, mol. sieves, 77%, 1.5:1 dr (**31**:**32**); (iii) Bu₃P, *o*-NO₂C₆H₄SeCN, THF, 89%; (iv) TAS-F, DMF/THF/H₂O (10/1/0.02), 0 °C, 98%; (v) TMSOOTMS, NaHCO₃, CH₂Cl₂, 65%; (vi) Bu₃P, *o*-NO₂C₆H₄SeCN, THF, 45%; (vii) TAS-F, DMF/THF/H₂O (10/1/0.02), 0 °C, 73%; (viii) TMSOOTMS, NaHCO₃, CH₂Cl₂, 68%.